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CONSERVATION AND REINTRODUCTION OF THE NATIVE ORCHIDS OF SINGAPORE

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ABSTRACT. Some 221 species of native orchids have been recorded in Singapore. Of these, however, ca. 170 orchid species are considered to be extinct, and only four are viewed as common. The orchid conservation program at the Singapore Botanic Gardens aims to monitor these species, to explore ways to conserve their germplasm, and to increase their number for subsequent reintroduction into appropriate habitats, including managed parks and roadsides. Thus far, we have successfully reintroduced *Grammatophyllum speciosum, Bulbophyllum vaginatum,* and *Bulbophyllum membranaceum.* Recently, we initiated the Orchid Cryo-Seed Bank Project, and have managed to successfully store seeds of four native species. They are *Dendrobium crumenatum, Spathoglottis plicata, Bulbophyllum vaginatum,* and *Dendrobium anosmun.*

Key words: conservation, reintroduction, seed bank, Singapore, native, orchids

INTRODUCTION

Singapore is located at ca. 1° north of the equator, off the southern tip of the Malay Peninsula between the South China Sea and the Indian Ocean. The nation consists of the main island of Singapore and 58 nearby islands. The total land area is ca. 690 km^2. The whole island consists of mostly lowland. The highest point is at Bukit Timah, which reaches an elevation of 165 m. The equatorial climate has a relatively uniform temperature and high humidity. Average daily temperatures fluctuate between 25.2°C and 32°C. Annual rainfall is ca. 1700 mm, with the wettest months November–January.

Singapore is a modern city, yet it has many interesting types of natural habitats. The heart of the main island has a primary rain forest and a freshwater swamp forest. In addition, some mangroves remain along the coast. Other habitats consist of secondary forests, shrub, grasslands, and urban parks and fields.

Of the 221 species of native orchids recorded in Singapore (Keng et al. 1998), more than 90% are endangered, vulnerable, rare, or extinct. This estimate is based on lists in a preliminary report on the conservation status of plants native to Singapore (Ng & Wee 1994, Tan 1995). The Singapore Botanic Gardens, therefore, has initiated an orchid conservation program to monitor these species, to attempt to find ways and means to conserve their germplasm, and to increase their number for subsequent reintroduction into appropriate habitats in nature reserves, parks, and roadside trees.

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METHODS: HABITS AND HABITATS

Native orchids in Singapore can be divided by plant habit into two main groups: epiphytes and terrestrials. They also can be grouped according to habitat, including open areas, semi-shade, and low-sunlight forest floors.

Epiphytes

No other genus better illustrates the epiphytic orchids of Singapore than the *Dendrobium* species, almost all of which are epiphytes. The largest genus among the native orchids, *Dendrobium* consists of 38 species (Tan 1984) or more than 20% of the total number of orchids in Singapore.

Dendrobium crumenatum. The pigeon orchid (*D. crumenatum*) is a well-known native species that can commonly be seen growing on the trunk of roadside trees. It is a delight to find them in bloom, as the flowers are sweetly scented. The species flowers 9–10 days after a heavy rainstorm. Similar flowering patterns have been observed in some native *Bulbophyllum, Thrixspermum, Bromheadia,* and *Taeniophyllum* species.

Dendrobium leonis section Aporum. Another interesting but rare native orchid, *D. leonis,* has leaves that are laterally flattened, thick and fleshy, and alternate in two rows. Individual yellowish-green flowers are borne near the apex of the stems. The flowers are only 1.5 cm across and have an extremely sweet vanilla-like fragrance that can be detected from a distance of 5 m.

Dendrobium aloifolium section Aporum. The native orchid *D. aloifolium* bears flowers 4 mm in diameter, probably the smallest in the section.

Dendrobium grande section Aporum. The native orchid *D. grande* has yellowish-green flowers, ca. 9 mm wide, tinted with red near the base of the sepals and petals, and the lip is deeply clefted.

Dendrobium anosmum (syn. *Dendrobium superbum*). Although widespread in Southeast Asia, this orchid species can no longer be found in Singapore. *Dendrobium anosmum* produces 1–4 large fragrant flowers at each node on leafless pendent stems. The flowers range in color from dark rose to deep lavender. The hairy lip is usually marked with deep purple stripes in the throat.

Bulbophyllum vaginatum section Cirrhopetalum. The second largest orchid genus in Singapore is *Bulbophyllum.* Species of this genus are divided into many sections. Of the 28 recorded species (Tan 1984), *B. vaginatum* is one of the most unusual. It grows in fairly exposed areas. Some plants grow naturally on branches of rain trees (*Samanea saman*) and on *Eugenia grandis* in the Singapore Botanic Gardens. The plants usually cover an extensive area of a branch and produce a spectacular sight when in full bloom. The flowers are creamy-yellow with ca. 15 of them arranged in a fan shape whorl or in a circle at the tip of the scape.

Bulbophyllum medusae. One of a number of interesting *Bulbophyllum* species in Singapore, *B. medusae,* another native orchid, resembles *B. vaginatum* but has paler and longer lateral sepals (12 cm).

Bulbophyllum membranaceum. In the Bukit Timah Nature Reserve, a clump of *B. membranaceum* is found creeping on a large piece of granite. Among the smallest native orchids, it has very small flowers ca. 6 mm long that do not open completely; the upper sepal is pale yellow, whereas lateral sepals are dark red, and the petals, 1.5 mm long, appear translucent. Other interesting *Bulbophyllum* of Singapore are *B. purpurescens, B. lepidum,* and *B. blumei.*

Vanilla griffithii. The *Vanilla* genus, among the most widely distributed in the world, has ca. 100 species distributed throughout the tropics, from Central and South America, across Central Africa, Southern India, Sri Lanka, and all of Southeast Asia to the Pacific Islands. In Singapore, the genus is represented by *V. griffithii,* a relatively common epiphytic climber found in the Upper Pierce Reservoir, Nee Soon, and the Mandai Forest. The plant climbs on tree trunks in a zigzag fashion. Petals and sepals of the flower are white, flushed with pale green, 2.5–3 cm long and 1.4–1.5 cm wide. The lip, white with a pale yellow tip, is hairy in the middle.

Cymbidium finlaysonianum. The most common of the three *Cymbidium* species in Singapore is *C. finlaysonianum.* Other species are *C. bicolor* var. *pubescens* and *C. aloifolium.* All native *Cymbidium* species are epiphytes.

Coelogyne pandurata. Among the four native species of the genus *Coelogyne, C. pandurata* is the most outstanding. The flower is ca. 6.5 cm across. Its petals and sepals have an attractive, clear apple-green color. The protruding lip has some conspicuous dark brown/red, almost black markings, hence, its common name, the black orchid.

Thrixspermum amplexicaule. Several species of the genus *Thrixspermum,* which is mostly epiphytic, have been recorded in Singapore. *Thrixspermum amplexicaule* (syn. *T. lilacinum*) is the most attractive, with a pale lilac-colored flower ca. 3 cm in diameter. The plant was first found in a semi-aquatic habitat in Singapore.

Thrixspermum trichoglottis. Also found in natural areas in Singapore, *T. trichoglottis* is very small with long seed capsules. The flowers are creamy yellow, with a brown spot on the lip. Among the other species of this genus are *T. calceolus, T. notabile,* and *T. ridleyanum.*

Grammatophyllum speciosum. Although Singapore is a small country, it is the home of the largest orchid plant in the world, *G. speciosum.* Also known as the tiger orchid, because of the markings on the flowers that resemble the skin of a tiger, it is extremely rare if not already extinct in the wild in Singapore. *Grammatophyllum speciosum* was last found in the wild in Tuas and Pulau Ubin (Ridley 1900). Since a mature plant could weigh more than a ton, it is a wonder that such a huge plant could live on the trunk of a tree.

Taeniophyllum obtusum. The extraordinary genus *Taeniophyllum,* the "leafless" orchid, has stems greatly reduced in size and covered by scale-like leaves. Roots are fleshy and spread over the bark of the host tree, usually on top of a thin layer of moss. These green roots replace leaves as the photosynthetic organ. As the plant is almost flat and its color blends very well with its host, one can hardly spot them in the wild. *Taeniophyllum obtusum* can be found near swampy areas in Singapore. The roots of the plant are ca. 2–3 mm wide. Flowering is gregarious, with the flower 5 mm wide; petals and sepals are orange-yellow, and the

lip is fleshy and concave, and white in color. Flowers only last for one day. Some of the *Taeniophyllum* species are known to produce a sweet fragrance that attracts pollinators.

Other beautiful native epiphytes include *Pomatocalpa latifolia, Arachnis hookeriana, Rhynchostylis gigantea, Renanthera histrionica, Phalaenopsis cornu-cervi, Liparis elegans, Liparis hyacinthoides, Eria javanica, Eria pudica, Eria pulchella, Dipodium pictum, Ariopsis javanica,* and *Flickingeria comata.* Some of these are extremely rare if not extinct in Singapore.

Terrestrials

Of the total number of native orchid species in Singapore, ca. 25% are terrestrials. These species can be found either in open areas or dwelling in the shade on forest floors.

Spathoglottis plicata. One of the most common terrestrial orchids in Singapore, *S. plicata,* frequently is found in open areas of the Bukit Timah Nature Reserve and the Central Catchment. It usually grows among grasses and ferns. The local species has two varieties: the dark pink and the alba forms. *Spathoglottis plicata* is very free flowering and has been used for landscaping.

Arundina graminifolia. Another common terrestrial species is *A. graminifolia.* Often found growing in habitats similar to those of *Spathoglottis plicata, A. graminifolia* is very free flowering in the lowland tropics. The vegetative parts of *A. graminifolia* resemble bamboo, which accounts for its common name, the bamboo orchid.

Bromheadia finlaysoniana. Among the native orchid species that prefer semi-shade, the familiar *B. finlaysoniana* is usually found along roadsides or on hill slopes. Like *Dendrobium crumenatum, B. finlaysoniana* flowers a few days after a rainfall. The flower is very attractive, faintly scented, white, with a yellow patch on the lip. Unfortunately, the flowers only last for a few hours.

Eulophia graminea. One of two species of the genus *Eulophia* found in Singapore, *E. graminea* is a very hardy plant. It grows among grasses and in partially shaded hill slopes and wastelands.

Eulophia squalida. Found in the Bukit Timah Nature Reserve growing on a rather steep slope, *E. squalida* has a flower with an unusual color. The sepals are brownish-olive and the petals white. The lip is pale mauve and has a white central area.

Nephelaphyllum pulchrum. Among the group of native orchids that dwell on the forest floor in Singapore, *N. pulchrum* thrives where the light intensity usually does not exceed 3000 lux during most of the day. Only occasionally do sunrays manage to penetrate the canopy to reach the forest floor. This native orchid has very interesting and showy leaves and delicate flowers. The leaves are fleshy; they are mottled light and dark green and flushed with purple. The flowers tend to crowd together on the short spike. The pinkish sepals and petals that all curve downwards are ca. 1.3 cm long.

Calanthe pulchra. Another forest-floor dweller, *C. pulchra* has striking orange flowers that do not open completely. The flowers are densely crowded along the 50–60 cm long inflorescence. A distinguishing feature of the flower is that its spur curves up like a hook. The leaves can reach a length of 70 cm and a width of 10 cm.

Plocoglottis javanica. Quite a common terrestrial orchid in Singapore, this inconspicuous forest-floor dweller has long pseudobulbs, which bear a single leaf. The leaves are 35 cm long by 10–12 cm wide. The long flower spike carries some yellow flowers that are spotted with red.

Cystorchis variegata. Unlike *Plocoglottis javanica, C. variegata* is a very rare species. This native forest-floor dweller has very attractive foliage. The leaves are arranged towards the apical part of the rhizome. They are light green in color with a darker network of venations. The half-opened flowers are borne on the elongated flower spike; the sepals are brown with a tinge of pink while the petals are white.

Claderia viridiflora. Also found on the forest floor of Singapore, *C. viridiflora* has a flower spike that bears a succession of many flowers, one or two at a time. The flowers are greenish, sepals and petals are ca. 2.5 cm long.

Zeuxine clandestine. Among the less-known orchids found in Singapore is *Z. clandestine.* Quite a large population of the species has been found in Bukit Timah, growing on the floor of a secondary forest. The plant is an inconspicuous creeper, with a fleshy rhizome. A young plant lies rather close to the ground, just like grass. When it begins to bloom, a flower spike of ca. 10–20 cm protrudes from the ground, bearing many small flowers. The flowers are light green in color and hardly open. The sepals are hairy, and ca. 3 mm long. The lip is a little shorter than the sepals.

Didymoplexis pallens. This species is an example of another group of terrestrial orchids that dwell on the forest floor but are mycotrophic (Arditti 1992). These orchids are parasitic on the fungi that associate with them. All orchids rely on mycorrhizae for their early growth, but the mycotrophic orchids depend on the fungus to provide them with nutrients throughout their life. These species usually dwell on the forest floor under dense canopy, in areas where little light can reach. *Didymoplexis pallens,* found in Bukit Timah Nature Reserve, has a fleshy underground rhizome; the flower spike is soft and slender, bearing several flowers. The flowers are pale brown-olive, with a tinge of pink, ca. 1.4 cm long. The dorsal sepal fuses to the petals for half its length; lateral sepals are also joined to the petals towards the base. The lip is light yellow, ca. 5 mm long. The species is not easily seen, because it has no visible vegetative part except for the erect flower spike, which hardly can be noticed in its natural habitat. If a flower is pollinated, however, the seed capsule can be seen in the wild. Owing to the difficulties in locating the plant, very little is known about this orchid. Last year, we also discovered another mycotropic orchid, *Leucanorchis multiflora,* at the Bukit Timah Nature Reserve.

Among the beautiful jewel orchid species recorded in Singapore are *Anoectochilus albolineatus* and *Macodes petola*; and examples of other native terrestrial orchids are *Corymborkis veratrifolia* and *Phaius tankervilleae.* Several forest-floor primitive species also can be found, such as *Apostatasia nuda* and *Neuwiedia veratrifolia.*

Methods and Materials

Based on lists in a preliminary report on the conservation status of plants native to Singapore (Ng & Wee 1994, Tan 1995), ca. 170 of Singapore's 221 species of native orchid species already are considered to be extinct, and only four are viewed as common. The remaining species are placed within the "Endangered," "Vulnerable," and "Rare" categories or have an indeterminate status. This means that more than 90% of the native orchids in Singapore are endangered, vulnerable, rare, or extinct.

A comparison of habitats on the Island of Singapore 150 years ago and today shows that most of the mangrove and marshy areas have been replaced by industrial estates or residential areas. Forest and mangrove areas, such as those at Choa Chu Kang, Jurong, Ang Mo Kio, and Serangoon, where native orchids used to thrive, no longer exist. Thus an orchid conservation program was initiated to monitor these species and to attempt to find ways and means to conserve their germplasm and to increase their numbers for subsequent reintroduction into appropriate habitats in the nature reserves, parks, and roadside trees. The strategies being explored by the Singapore Botanic Gardens are (1) propagation and reintroduction, and (2) cryo-seed banks.

Conservation and Reintroduction

First, we want to propagate species vegetatively and by in-vitro culture. To increase the population of some of the rarer species, which will be either selfed or sibbed, seeds are germinated in vitro (Yam & Weatherhead 1988), and seedlings are introduced back to nature. So far, we have managed to propagate a number of orchid species and have reintroduced three species to nature areas in Singapore: *Grammatophyllum speciosum,* the tiger orchid; *Bulbophyllum vaginatum*; and *B. membranaceum.*

H.N. Ridley, an early director of the Singapore Botanic Gardens, recorded that the tiger orchid was found in the wild in Toas (Tuas) and Pulau Ubin (Ridley 1900). Unfortunately, naturally occurring plants are now extinct.

A few years ago, a tiger orchid growing in the Gardens flowered and was self-pollinated. The huge seedpod was harvested 7 months later. Seeds germinated a month after being sown on Knudson C medium (Knudson 1946). After 12 months in the laboratory, the seedlings were planted out in the nursery.

Since the tiger orchid last occurred naturally in Pulau Ubin, the first batch of seedlings was reintroduced there in July 1999, when the seedlings were 26 months old and ca. 15–20 cm tall with 5–6 leaves. They were affixed on durian, rambutan, mango, angsana, tembusu, and rain trees. Seedlings also were planted on trees in the Botanic Gardens, around the Visitor Centre at the Bukit Timah Nature Reserve, and in the Orchard Boulevard area in the heart of the city.

With experience from the initial trials, we decided to introduce seedlings to the Bukit Batok Nature Park in early 2001. This time, the seedlings were more mature, ca. 30–40 cm tall with 16–20 leaves. They had at least three shoots, a well-established root system, and fleshy pseudobulbs. In February 2001, these larger seedlings were planted on trees along Orchard Boulevard and on the yellow rain trees around the Bandstand in the Botanic Gardens. In April 2001, seedlings of the same age were introduced to a site adjacent to a mangrove area in Pulau

TABLE 1. Survival rate of reintroduced seedlings of *Grammatophyllum speciosum,* the tiger orchid, to various parts of Singapore.

Location	Seedling size cm	Survival rate %
Pulau Ubin	15–20	35
	30–40	90
Bukit Timah Nature Reserve	15–20	10
	30–40	75
Botanic Gardens	15–20	10
	30–40	70
Orchard Boulevard	15–20	45
	30–40	90
Bukit Batok Nature Park	30–40	90
Holland Road	30–40	95
Upper Pierce Reservoir	30–40	95

Ubin, and in early May 2001 again to the Bukit Timah Nature Reserve. Some 40 seedlings also were planted on rain trees along Holland Road. In December 2002, some 40 seedlings were planted on trees at the Upper Pierce Reservoir. In early 2004, some 20 plants were planted at various urban parks, such as Sembawang Park, Kent Ridge Park, and Tiong Bahru Park. Our aim is to use native orchids to educate the public about our rich natural heritage and our responsibility to conserve it.

The tiger orchid seedlings we planted have been growing for 2–5 years in their new homes, and a majority of them are doing well. New shoots have developed, and roots are firmly established on tree trunks. Unfortunately, most seedlings planted in the Bukit Timah Nature Reserve were damaged or removed by animals (probably by squirrels and monkeys respectively) (TABLE 1).

Several factors appear to play major roles in the survival of introduced seedlings. These include the microclimate of the area (relative humidity, for example), texture of the bark of host trees, presence of other epiphytes, and the size of the seedlings.

Seedlings planted in areas with high relative humidity tend to survive better than those in dry areas. For example, in Pulau Ubin, seedlings established in a damp area inside a secondary forest are healthier and more vigorous than those growing near the sea where the breeze tends to dry the bark faster. Texture of the bark is important because certain barks tend to retain more moisture. For instance, rain trees are generally better hosts than tembusu. Trees that support more epiphytes tend to be better hosts than those with fewer epiphytes. It seems that if the conditions are suitable for other epiphytes, they also are more appropriate for *Grammatophyllum speciosum.* The size of seedlings (TABLE 1) also is a key factor in determining survival. Seedlings with 16–20 leaves (30–40 cm tall) tend to survive better than those with only five leaves (15–20 cm tall).

We hope that the tiger orchids will continue to thrive in their new homes. In the near future, more of this beautiful and majestic species will find its way to more nature areas and parks in Singapore.

In 2003 and 2004, two other native orchids, *Bulbophyllum vaginatum* and *B. membranaceum* were successfully propagated and reintroduced. Seeds of these species were collected from plants growing in their natural habitats. The seeds were sown on Knudson C medium. Seedlings were grown on the media until 2–3 cm tall before being transferred to the nursery. Some ten seedlings were planted on fern barks measuring 7 cm long by 5 cm wide and grown at the nursery for 6 months, until new shoots began to develop. They were then reintroduced. Host trees were selected based on the same criteria used for reintroducing the tiger orchid. When a suitable tree is selected, fern barks with established seedlings were secured on the tree trunk by nails.

Thus far, some 500 seedlings of *Bulbophyllum vaginatum* and *B. membranaceum* have been reintroduced. More than 90% have settled down and are growing well in their new homes. We have learned that these seedlings are best planted in slightly shady areas, with at least 50% shade to avoid being scorched. We are very pleased to report that most of these seedlings have produced new shoots and are growing onto the bark of the host tree.

Orchid Cryo-Seed Bank

The second conservation strategy is to store orchid seeds under liquid nitrogen as part of an Orchid Cryo-Seed Bank Project. Why do we store seeds in liquid nitrogen? Although tropical orchids have been grown routinely from seed for many years, using the asymbiotic method for seed germination, relatively little progress has been made in developing techniques for storing the seeds. Storage is becoming increasingly important, however, in view of the rapid loss of many species caused by habitat destruction and over-collection. The reason for storing seeds under such low temperature is simple. When plant and animal tissues are stored at ultra-low temperatures, such as that of liquid nitrogen (−196°C), metabolism is effectively stopped and cells are preserved without deterioration until such time as they are thawed for use.

There are several reasons for the cryopreser-

vation of orchid seeds: (1) Seed storage ensures against failure to germinate or the accidental loss of seedlings. (2) Instead of keeping hundreds of plants in the nursery, thousands of orchid seeds (which are very small), can be stored in a small container. If stored under the proper conditions, the seeds can be kept for years under such conditions. The seeds can be sown as and when the need arises. The seeds also can be distributed to (or exchanged with) other botanic institutions for conservation. (3) Seeds of hybrids can be stored as well, once the technique is perfected. This will allow assessment of the commercial merits and potential of a particular cross while still retaining a proportion of the seeds. (4) Plant germplasm collections form an integral part of all plant-breeding programs. The purpose of germplasm preservation is to ensure the availability of useful germplasm at any time. Germplasm storage in the form of seeds is economical and efficient.

During the last 2 years, we carried out a series of experiments to investigate the optimal conditions for storing some native orchid seeds. We studied the effects of pre-storage treatment such as desiccation, effectiveness of various desiccants, duration of desiccation, age of seeds, and different types of containers.

Thus far, we have managed to successfully store seeds of four native species. They are *Dendrobium crumenatum, Spathoglottis plicata, Bulbophyllum vaginatum,* and *Dendrobium anosmun.* The first three species are still common in Singapore. The last one can no longer be found in Singapore. To carry out the systematic investigations, we need a large quantity of seeds; hence the three common species were used. Some of the seeds have been stored under liquid nitrogen for more than 24 months. Recently, we attempted to germinate the stored seeds and are pleased to report that more than 90% of the seeds remained viable. This gives us a great sense of excitement. Seeds of tropical orchids can be preserved under −196°C after all! From the results of the experiment, we find the best procedure for storing the seeds that we have tested is as follows: (1) Collect fresh seeds from healthy, mature seed capsules. (2) Desiccate the seed for several days in a desiccator that contains a saturated solution and calcium chloride. (3) Transfer a small quantity of dried seeds to a 2-ml cryogenic vial. (4) Store the vial in a liquid nitrogen freezer.

To conclude, although remarkable progress has been made, cryobiological storage remains a relatively new science, but one which holds promise for the long-term storage of germplasm. The freezing of plant samples at a low temperature is still in a highly empirical state. Cryopreservation offers a practical, efficient, and economical tool for long-term storage of germplasm. Tropical species cryopreservation has not been studied extensively, because these plants are known to be more sensitive to chilling, low temperature, and desiccation than are temperate species. Therefore, a considerable amount of research still needs to be conducted, before we can use the technique confidently for long-term preservation of orchid seeds. At the moment, we are experimenting to fine-tune the storage procedure and determine if we can use it to store seeds of other species and hybrid orchids.

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